Review

Hemotrophic mycoplasmas, occurrence and detection methods in animals of veterinary importance

Micoplasmas hemotrópicos, presencia y métodos de detección en animales con importancia veterinaria

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ABSTRACT: Hemotrophic mycoplasmas (hemoplasmas) pose a threat to animal health. During the last years, the presence of hemoplasmas around the world has significantly increased thanks to recent molecular detection methods that have been developed. Hemoplasmas cause severe alterations in the health of the host animal, either alone or in co-infection. These include cats, dogs, mice, pigs, and cattle. They cause severe anemia to chronic infection, clinical signs for which the animal may eventually die. Current interest in hemoplasmas is based on their pathogenic role at the molecular level in the host animal. This review presents information on hemoplasmas diversity and the potential for them to be considered a zoonotic risk. It also highlights the development of several molecular techniques for the diagnosis of hemoplasmas, which allow a quick and accurate detection to make decisions in case of an infection event.

Key words: hemoplasmas diversity, phylogenetic reconstruction, diagnosis, co-infections.

RESUMEN: Los micoplasmas hemotróficos (hemoplasmas) representan una amenaza para la salud animal. Durante los últimos años, la presencia de hemoplasmas alrededor del mundo se ha incrementado significativamente gracias a los recientes métodos de detección moleculares que se han desarrollado. Los hemoplasmas causan alteraciones severas en la salud del animal hospedador, ya sea solos o en coinfección. Entre estos se incluyen los gatos, perros, ratones, cerdos y el ganado bovino. Causan anemia severa hasta una infección crónica, signos clínicos por los que eventualmente el animal puede morir. El interés actual en los hemoplasmas se basa en su papel patogénico a nivel molecular en el animal hospedador. En esta reseña se presenta la información sobre la diversidad de los hemoplasmas y el potencial para ser considerados como riesgo zoonótico. Se resalta también el desarrollo de diversas técnicas moleculares para el diagnóstico de los hemoplasmas, que permiten una detección rápida y precisa para tomar decisiones ante un evento de infección.

Palabras clave: diversidad de hemoplasmas, reconstrucción filogenética, diagnóstico, coinfección.

INTRODUCTION

The interest in hemotrophic mycoplasmas (also called hemoplasmas) is growing worldwide, mainly due to the detection of hemoplasmas by molecular methods. Either alone or in co-infection with other microorganisms, hemoplasmas are associated with clinical signs in domestic and wild animals (1,2). In addition, hemoplasmas can act as opportunistic agents that silently infect animals and humans (3).

Hemoplasmas are small, Gram-negative and cell wall-less bacteria, considered to be obligate erythrocyte bacteria, which up to now have been uncultivable, in contrast to mucosal mycoplasmas (3). Hemoplasmas are pleomorphic (cocci, rods, rings), 0.3 to 1 µm in diameter, with small genomes (0.5-1.0 Mb). They are usually attached to the outer surface of the red blood cells forming slits (3).

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Hemoplasmosis can cause hematological disorders in several mammalian species, ranging from severe anemia to chronic infection without clinical signs. Those animals with acute infections may present hemolysis, anorexia, dehydration, fever, loss of weight, lethargy, and even sudden death (3).

The transmission of hemoplasmosis mediated by different vectors depending on the pathogen. Fleas, ticks, lice, and flies are responsible for the transmission of hemoplasmas in cats, dogs, mice, pigs, and cattle (4,5). Although hemoplasmosis is not strictly considered a tick-borne disease, they may play a role in the epidemiology of these bacteria as some hemoplasmas are occasionally detected in cattle ticks (6).

HEMOPLASMAS CLASSIFICATION AND PHYLOGENETIC ANALYSES

Based on the morphological characteristics, response to antibiotics, Gram-negative staining, erythrocyte tropism, and putative arthropod transmission, hemoplasmas were initially classified in the order Rickettsiales, family Anaplasmataceae and genera *Eperythrozoon* and *Haemobartonella*. Currently, based on their 16S rRNA gene sequence, they have been reclassified from the genus *Rickettsia* to the genus *Mycoplasma* since phylogenetic reconstruction shows a robust relationship with members of the Mycoplasmataceae family (3,7,8). The genus *Mycoplasma* groups microorganisms that can establish commensal or virulent or both relations with the host

The phylogenetic reconstruction of hemoplasmas is based on the 16S rRNA gene and the RNA subunit of the RNAase P (*rnpB*) gene (9-12). These phylogenies are important tools to classify new hemoplasma species and to denote the group of hemoplasmas from the rest of *Mycoplasma* groups (9). Hemoplasmas are present in domestic and wild animals, including cats, dogs, bovines, buffaloes, mice, sheep, goats, feral cats, among others. Both genome sequencing of some hemoplasmas (so far, existing 11 hemoplasma genomes) and 16S rRNA sequences allowed the identification of hemoplasmas of veterinary health importance, many of them named as *Candidatus* (*Ca.*) since they are uncultivable (13-15).

HEMOPLASMAS IN COMPANION ANIMALS

Hemoplasmas of felines are widely reported, including Mycoplasma haemofelis, *Candidatus* Mycoplasma haemominutum, *Candidatus* Mycoplasma haematoparvum, and *Candidatus* Mycoplasma turicensis (4,16-18). These four species produce hemolytic anemia in cats; however, *M. haemofelis* is the most pathogenic. *Ca.* M. haemominutum and *Ca.* M. turicensis may induce anemia when the host is immunosuppressed or when a concurrent disease is present, for instance, those caused by the feline leukemia virus (FeLv) (18,19).

Clinical signs caused by feline hemoplasmas include anemia, pallor, lethargy, anorexia, weight loss, pyrexia, and dehydration. Tetracyclines or fluoroquinolones are an effective treatment, although the infection may persist (18).

The most common hemoplasma species in dogs are *Mycoplasma haemocanis* and *Candidatus* Mycoplasma haematoparvum (20); other dog hemoplasmas are *Candidatus* M. haemominutum and *Candidatus* M. turicensis (21,22).

HEMOPLASMAS IN PRODUCTION ANIMALS

Usually, cattle infected with hemoplasmas look healthy. In Mexico, *Candidatus* Mycoplasma haemobos and *Mycoplasma wenyonii* were recently identified in cattle and their genomes were reported (23,24). Brazilian studies on the detection and occurrence of *Ca* .M. haemobos in cattle revealed that infected animals might represent chronically asymptomatic carriers with the risk of transmission to those healthy animals (12). In some cases of females and calves infection, symptoms such as transient fever, anorexia, lymphadenopathy, decreased milk yield, and weight loss, are observed (25).

The detection of *Ca.* M. haemobos and *M. wenyonii* in sick animals during a fatal anaplasmosis outbreak in Switzerland suggested that co-infection of both hemoplasmas could increase their pathogenicity in cattle (26). In

cattle, the prevalence of these hemoplasmas is higher in adults and rare in calves, perhaps due to the immune protection of the mother.

Swine hemoplasmas *Mycoplasma suis* and *Mycoplasma parvum* parasitize the surface of red the blood cells causing membrane deformations and damage, which lead to anemia and icterus in pigs. The adverse effects of hemoplasmas in pigs include decreased reproductive efficiency in sows, delayed estrus, early embryonic death, and late-term abortion; newborn and weaned piglets shows severe anemia and pyrexia (27).

M. parvum is a nonpathogenic bacterium of pigs. It is often accumulated on the red blood cells infecting only a few cells. Frequently, this pathogen is unnoticeable in the absence of clinical signs even in splenectomized pigs (28). The comparative genomic analyses have shown the different pathogenicity levels between *M. suis* and *M. parvum* (29), and the similarities in the number of coding DNA sequences (CDS) related to metabolic functions, transporters, and putative virulence factors.

The molecular epidemiology of sheep and goat hemoplasmas is poorly studied. *Mycoplasma ovis* and *Candidatus* Mycoplasma haemovis are the two species identified in these animals (30). *M. ovis* infecting reindeers (*Rangifer tarandus*) causes weight loss and moderate anemia, among other symptoms (31).

HEMOPLASMAS CO-INFECTIONS

Infectious agents are continually threatening animal health, and often they establish relationships with other pathogens that might severely impact the infection process. This interaction between pathogens may be useful as mobility support, enhanced contagiousness, and accelerated virulence. (32). In hemoplasmas, the role of each pathogen during a co-infection and their molecular interactions are still unknown. Table 1 shows several co-infections reported in animals.

DETECTION OF HEMOPLASMAS

In the last ten years, the number of reports related to hemoplasmosis in different hosts increased significantly due to the use of molecular detection methods.

The detection of hemoplasmas includes the detection of specific antibodies, microscopic visualization of the organisms, and more recently, molecular-based methods. Giemsa staining and acridine orange are the most widely used methods for visualizing hemoplasmas, providing information on the presence of the pathogen but not its identity (33).

The electron microscopy is a specific technique used for the observation of *M. suis* in infected tissues (34). Besides the scanning electron microscopy allows observing the replication and attachment of hemoplasmas to erythrocytes as well as the damage they cause to the endothelial cells and other host tissues.

In situ hybridizations of fixed tissue sections allowed locating the attachment site of *M. haemofelis* to the liver, kidney cells and red blood cells (35). In this case, besides visualizing, the speciation of the causal agent was also attained.

Pathogen detection is the first step to characterize the agent or agents of a co-infected host. Especially, PCR-based tests of the 16S

Table 1. Hemoplasmas co-infections reported in animals./*Co-infecciones de hemoplasmas reportadas en animales.*

Co-infection	Hemoplasmas	Reference
Dogs	*M. haemocanis* and *Ca.* M. haematoparvum	(40)
	M. haemocanis and *Anaplasma platys*	(40)
	Ca. M. haematoparvum and *A. platys*	(40)
	M. haemocanis and *Babesia vogeli*	(40)
	M. haemocanis and *Ehrlichia canis*	(1)
	Babesia conradae, Ca. M. haematoparvum, *M. haemocanis*	(41)
Cats	*Ca.* M haemominutum, *M. haemofelis* or *Bartonella henselae*	(42)
Dairy cattle and Water Buffalo	*Mycoplasma wenyonii* and *Ca.* M. haemobos	(43,44)

rRNA gene followed by sequencing is the most used molecular method to detect and identify hemoplasma species. New hemoplasma genomes allow finding sequences to design specific primers for proper identification, besides 16S rRNA PCR followed by restriction fragment length polymorphism (RFLP), which has been successfully used for the diagnosis of *M. haemofelis* and *M. haemominutum* in cats (36).

Real-Time TaqMan or SYBR green PCR assays have some advantages over more traditional detection methods as they allow quantification, have minimal risk of amplicon carryover and are highly specific. However, due to their specificity, these assays are unlikely to detect novel hemoplasma species (37).

Diagnostic methods that permit fast differentiation are necessary when a limited number of hemoplasmas are suspected. Thus, the analysis of the melting curve of SYBR green-based RT-PCR products is an excellent tool to differentiate between hemoplasma species. For instance, hemoplasma prevalence in the Miyagi Prefecture of 109 bovine cattle was as follows: 67 animals (61.5 %) infected with *M. wenyonii*, 25 animals (22.9 %) infected with *Ca.* M. haemobos, and 14 (12.8 %) infected with both (38). A similar method was used for feline hemoplasmosis with encouraging results as the authors discerned among seven different mycoplasmas from the blood of suspect cats and other felines (39).

Undoubtely, the combination of several detection methods enhances the possibility to confirm the presence of hemoplasmas. For instance, the propidium iodide staing of blood smears and the end-point PCR for the detection of *Ca*. M. haemobos and *M. wenyonii* confirm the presence of both pathogens in Mexican cattle (Fig. 1). Diagnostic methods allowing a fast differentiation are necessary when a limited number of hemoplasmas are suspected. Table 2 shows a summary of primers and techniques used for hemoplasmas identification.

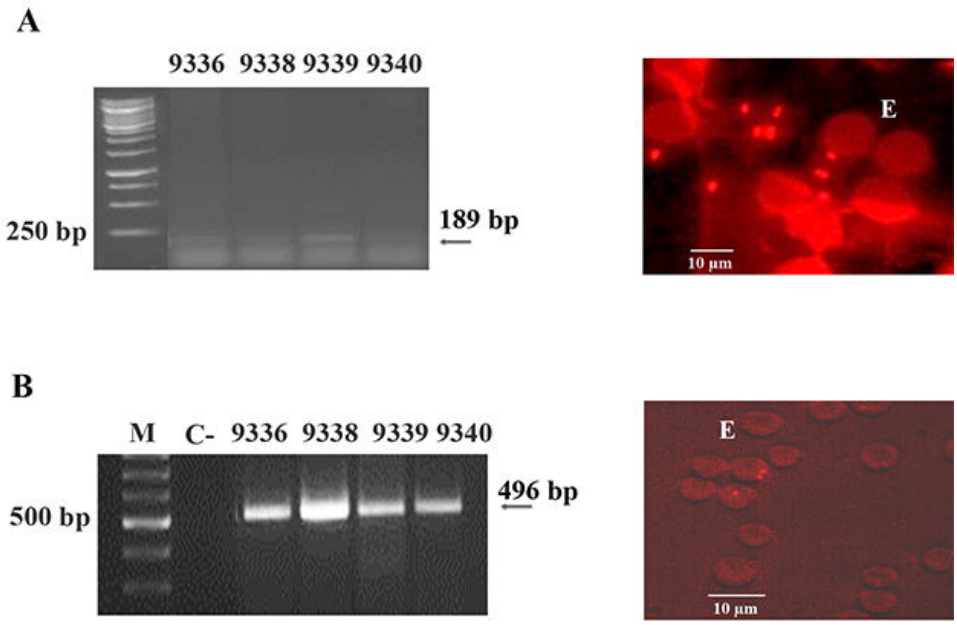

Figure 1. Micrography of staining of (A) *Ca*. M. haemobos and (B) *M. wenyonii* with propidium iodide correlates with the end-point PCR detection of both pathogens in Mexican cattle. Micrography of *Ca.* M. haemobos taken from (52)/*Micrografía de la tinción de Ca. M. haemobos and M. wenyonii con yoduro de propidio y detección de ambos patógenos por PCR punto final en muestras de sangre de ganado mexicano. Micrografía de Ca. M. haemobos tomada de* (52).

Table 2. Oligonucleotide sequences used for identification by molecular methods./*Secuencias de oligonucleótidos empleados para su identificación por métodos moleculares.*

Hemoplasma species (Host)	Primer sequences	Ref.
End-Point PCR/sequencing 16S RNA/ITS sequence		
Ca. M. haemobos (bovine)	F: 5'-GCATCTAGAGTGAACATTCTGATTGG-3'	(44)
	R:5'CCTAGCTTATCGCAGATTAGCACGT-3'	
Ca. M. haemobos (bovine)	F 5'-ATCTAACATGCCCCTCTGTA-3'	(38)
	R 5'-GTAGTATTCGGTGCAAACAA-3'	
M. wenyonii (bovine)	F 5'-ACTTTTACGAGGAGGATAGC-3'	
	R 5'-TGATTAACTCTAGGGAGGCG-3'	
Ca. M. haemobos (cattle)	F 5'-ATATGGCCCATATTCCTACG-3'	(12,45)
	R 5'-TGCTCCACCACTTGTTCA-3'	
M. wenyonii; Ca. M. haemobos (bovine, goats)	F 5'-GGCCCATATTCCT(AG)CGGGAAG-3'	(46,47)
	R 5'-AC(AG)GGATTACTAGTGATTCCA-3'	
M. haemofelis, M. haemominutum (felines)	F 5'-ATACGGCCCATATTCCTACG-3'	(36)
	R 5'-TGCTCCACCACTTGTTCA-3'	
M. haemofelis; Ca. M. turicensis (felines)	F 5'-GTATCCTCCATCAGACAGAA-3'	(48)
	R 5'-CGCTCCATATTTAATTCCAA-3'	
M. suis, M. parvum (swine)	F 5'-TAAATTAAAGGAGGCTGCCGMAAGGTG-3'	(49)
	R 5'-TACGCCCAATAAATCCGGATAATGCTC-3'	
M. hemocanis; *Ca*. M. hematoparvum (dogs)	F 5'-ATACGGCCCATATTCCTACG-3'	(50)
	R 5'-TGCTCCACCACTTGTTCA-3'	
rtPCR, SYBR green/sequencing of fragments		
Ca. M. haemominutum *M. haemofelis* (felines)	F 5'-GAAAGTCTGATGGAGCAATACCAT-3'	(51)
	R 5'-CTGGCACATAGTTWGCTGTCACTTA-3'	
	F 5'-GAAAGTCTGATGGAGCAATACCAT-3'	
	R 5'-CATAGTTWGCTGTCACTTA-3'	

CONCLUSION

The distribution of hemoplasmas comprises different hosts. Over time, hemoplasmas found either alone or in co-infections become a risk to companion and production animals and even to human health. The absence or presence of clinical signs in infected animals could be related to the interactions established by the pathogens in the host. Hence, the importance to develop more precise molecular tools for early diagnoses.

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REFERENCES

1. Kaewmongkol G, Lukkana N, Yangtara S, Kaewmongkol S, Thengchaisri N, Sirinarumitr T, et al. Association of Ehrlichia canis, hemotropic Mycoplasma spp. and Anaplasma platys and severe anemia in dogs in Thailand. Vet Microbiol. 2017; 201:195-200.
2. Maggi RG, Mascarelli PE, Havenga LN, Naidoo V, Breitschwerdt EB. Co-infection with Anaplasma platys, Bartonella henselae and Candidatus Mycoplasma haematoparvum in a veterinarian. Parasit Vectors . 2013; 15;6:103. Available from: http://

www.ncbi.nlm.nih.gov/pmc/articles/PMC3637287/

3. Messick JB. Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. Vet Clin Pathol 2004; 33(1):2-13. Available from: http://doi.wiley.com/10.1111/j.1939-165X.2004.tb00342.x
4. Willi B, Novacco M, Meli M, Wolf-Jackel G, Boretti F, Wengi N, et al. Haemotropic mycoplasmas of cats and dogs: transmission, diagnosis, prevalence and importance in Europe. Schweiz Arch Tierheilkd. 2010;152(5):237-44.
5. Hornok S, Foldvari G, Elek V, Naranjo V, Farkas R, de la Fuente J. Molecular identification of Anaplasma marginale and rickettsial endosymbionts in blood-sucking flies (Diptera: Tabanidae, Muscidae) and hard ticks (Acari: Ixodidae). Vet Parasitol. 2008;154(3-4):354-9.
6. Barker EN, Tasker S, Day MJ, Warman SM, Woolley K, Birtles R. Development and use of real-time PCR to detect and quantify Mycoplasma haemocanis and `Candidatus Mycoplasma haematoparvum' in dogs. Vet Microbiol 2010;140. Available from: http://dx.doi.org/10.1016/j.vetmic.2009.07.006
7. Rikihisa Y, Kawahara M, Wen B, Kociba G, Fuerst P, Kawamori F, et al. Western immunoblot analysis of Haemobartonella muris and comparison of 16S rRNA gene sequences of H. muris, H. felis, and Eperythrozoon suis. J Clin Microbiol. 1997;35(4):823-9.
8. Neimark H, Johansson K-. E, Rikihisa Y, Tully JG. Proposal to transfer some members of the genera Haemobartonella and Eperythrozoon to the genus Mycoplasma with descriptions of Candidatus Mycoplasma haemofelis, Candidatus Mycoplasma haemomuris, Candidatus Mycoplasma haemosui' and Candidatus Mycopl. Int J Syst Evol Microbiol 2001;51. Available from: http://dx.doi.org/10.1099/00207713-51-3-891
9. Peters IR, Helps CR, McAuliffe L, Neimark H, Lappin MR, Gruffydd-Jones TJ, et al. RNase P RNA gene (rnpB) phylogeny of hemoplasmas and other mycoplasma species. J Clin Microbiol. 2008; 1;46(5):1873-7. Available from: http://jcm.asm.org/content/46/5/1873.abstract
10. Tasker S, Helps CR, Day MJ, Harbour DA, Shaw SE, Harrus S, et al. Phylogenetic analysis of hemoplasma species: an international study. J Clin Microbiol. 2003 1;41(8):3877-80. Available from: http://jcm.asm.org/content/41/8/3877.abstract
11. Congli Y, Hong Y, Zhonghai Z, Zhibiao Y, Li C, Jianguo Z, et al. Prevalence of Mycoplasma wenyonii infection on seven dairy farms in Shanghai, China. Thai J Vet Med. 2011;41(2):179-84.
12. Girotto A, Zangirolamo AF, Bogado ALG, Souza ASLE, da Silva GCF, Garcia JL, et al. Molecular detection and occurrence of "Candidatus Mycoplasma haemobos" in dairy cattle of Southern Brazil. Rev Bras Parasitol Vet. 2012;21(3):342-4.
13. Cubilla MP, Santos LC, de Moraes W, Cubas ZS, Leutenegger CM, Estrada M, et al. Occurrence of hemotropic mycoplasmas in non-human primates (Alouatta caraya, Sapajus nigritus and Callithrix jacchus) of southern Brazil. Comp Immunol Microbiol Infect Dis. 2017;52:6-13.
14. Messick JB, Santos AP, Guimaraes AMS. Complete genome sequences of two hemotropic mycoplasmas, Mycoplasma haemofelis strain Ohio2 and Mycoplasma suis Strain Illinois. J Bacteriol. 2011;193(8):2068-9.
15. Willi B, Tasker S, Boretti FS, Doherr MG, Cattori V, Meli ML. Phylogenetic analysis of `Candidatus Mycoplasma turicensis' isolates from pet cats in the United Kingdom, Australia, and South Africa, with analysis of risk factors for infection. J Clin Microbiol. 2006;44. Available from: http://dx.doi.org/10.1128/JCM.00987-06
16. Reynolds CA, Lappin MR. `Candidatus Mycoplasma haemominutum' infections in 21 client-owned cats. J Am Anim Hosp Assoc. 2007;43. Available from: http://dx.doi.org/10.5326/0430249
17. Willi B, Filoni C, Catão-Dias JL, Cattori V, Meli ML, Vargas A, et al. Worldwide cccurrence of feline hemoplasma infections in wild felid species. J Clin Microbiol. 2007 14;45(4):1159-66. Available from: http://

www.ncbi.nlm.nih.gov/pmc/articles/PMC1865832/

18. Tasker S. Haemotropic mycoplasmas: What's their real significance in cats? J Feline Med Surg. 2010; 12(5):369-81. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2880789/
19. Willi B, Boretti FS, Cattori V, Tasker S, Meli ML, Reusch CE. Identification, molecular characterization, and experimental transmission of a new hemoplasma isolate from a cat with hemolytic anemia in Switzerland. J Clin Microbiol. 2005;43. Available from: http://dx.doi.org/10.1128/JCM.43.6.2581-2585.2005
20. Wengi N, Willi B, Boretti FS, Cattori V, Riond B, Meli ML, et al. Real-time PCR-based prevalence study, infection follow-up and molecular characterization of canine hemotropic mycoplasmas. Vet Microbiol. 2008;126(1-3):132-41.
21. Sykes JE, Ball LM, Bailiff NL, Fry MM. "Candidatus Mycoplasma haematoparvum", a novel small haemotropic mycoplasma from a dog. Int J Syst Evol Microbiol. 2005;55(Pt 1):27-30.
22. Obara H, Fujihara M, Watanabe Y, Ono HK, Harasawa RJ. A feline hemoplasma, `Candidatus Mycoplasma haemominutum', detected in dog in Japan. J Vet Med Sci. 2011;73. Available from: http://dx.doi.org/10.1292/jvms.10-0521
23. Martínez-Ocampo F, Rodríguez-Camarillo SD, Amaro-Estrada I, Quiroz-Castañeda RE. Draft genome sequence of "Candidatus Mycoplasma haemobos," a hemotropic mycoplasma identified in cattle in Mexico. Genome Announc. 2016; 25;4(4). Available from: http://genomea.asm.org/content/4/4/e00656-16.abstract
24. Quiroz-Castañeda RE, Martínez-Ocampo F, Dantán-González E. Draft Genome Sequence of Mycoplasma wenyonii, a second hemotropic Mycoplasma Species Identified in Mexican Bovine Cattle. Stajich J, editor. Microbiol Resour Announc. 2018;7(9).
25. Witter R, Melo ALT, Pacheco T dos A, Meneguzzi M, Vilas Boas R, Nakazato L, et al. Prevalence of 'Candidatus Mycoplasma haemobos' detected by PCR , in dairy cattle from Ji-Paraná in the north region of Brazil. Ciência Rural. 2017;47(3):e20160805.
26. Meli ML, Willi B, Dreher UM, Cattori V, Knubben-Schweizer G, Nuss K, et al. Identification, molecular characterization, and occurrence of two bovine hemoplasma species in Swiss cattle and development of real-time TaqMan quantitative PCR assays for diagnosis of bovine hemoplasma infections. J Clin Microbiol. 2010;48(10):3563-8.
27. Tagawa M, Yamakawa K, Aoki T, Matsumoto K, Ishii M, Inokuma H. Effect of Chronic Hemoplasma Infection on Cattle Productivity. J Vet Med Sci. 2013; 15;75(10):1271-5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3942926/
28. Splitter, E J. Eperythrozoon suis n. sp. and Eperythrozoon parvum n. sp., 2 new blood parasites of swine. Science. 1950;111(2889):513-4.
29. do Nascimento NC, dos Santos AP, Chu Y, Guimaraes AMS, Baird AN, Weil AB, et al. Microscopy and genomic analysis of Mycoplasma parvum strain Indiana. Vet Res. 2014; 13;45(1):86. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4423628/
30. Wang X, Cui Y, Zhang Y, Shi K, Yan Y, Jian F, et al. Molecular characterization of hemotropic mycoplasmas (Mycoplasma ovis and `Candidatus Mycoplasma haemovis') in sheep and goats in China. BMC Vet Res. 2017;13(1):142. Available from: https://doi.org/10.1186/s12917-017-1062-z
31. Stoffregen WC, Alt DP, Palmer M V, Olsen SC, Waters WR, Stasko JA. Identification of a haemomycoplasma species in anemic reindeer (Rangifer tarandus). J Wildl Dis. 2006;42(2):249-258.
32. Wade MJ. The co-evolutionary genetics of ecological communities. Nat Rev Genet. 2007;8(3):185-195.
33. Jasper DE, Jain NC. Histochemical observations on Mycoplasma after staining with acridine orange. Appl Microbiol. 1966;14(5):720-723.
34. Schreiner SA, Hoelzle K, Hofmann-Lehmann R, Hamburger A, Wittenbrink MM, Kramer MM, et al. Nanotransformation of the

haemotrophic Mycoplasma suis during in vitro cultivation attempts using modified cell free Mycoplasma media. Vet Microbiol. 2012;160(1-2):227-232.

35. Berent LM, Messick JB, Cooper SK, Cusick PK. Specific in situ hybridization of Haemobartonella felis with a DNA probe and tyramide signal amplification. Vet Pathol. 2000;37(1):47-53.
36. Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC. Presence of Mycoplasma haemofelis, Mycoplasma haemominutum and piroplasmids in cats from southern Europe: a molecular study. Vet Microbiol. 2003;93(4):307-17.
37. Hoelzle LE, Helbling M, Hoelzle K, Ritzmann M, Heinritzi K, Wittenbrink MM. First LightCycler real-time PCR assay for the quantitative detection of Mycoplasma suis in clinical samples. J Microbiol Methods. 2007;70(2):346-354.
38. Nishizawa I, Sato M, Fujihara M, Sato S, Harasawa R. Differential Detection of Hemotropic Mycoplasma Species in Cattle by Melting Curve Analysis of PCR Products. J Vet Med Sci. 2010;72(1):77-79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19893280
39. Willi B, Meli ML, Lüthy R, Honegger H, Wengi N, Hoelzle LE. Development and application of a universal hemoplasma screening assay based on the SYBR Green PCR principle. J Clin Microbiol. 2009;47. Available from: http://dx.doi.org/10.1128/JCM.01478-09
40. Mascarelli PE, Tartara GP, Pereyra NB, Maggi RG. Detection of Mycoplasma haemocanis, Mycoplasma haematoparvum, Mycoplasma suis and other vector-borne pathogens in dogs from Cordoba and Santa Fe, Argentina. Parasit Vectors. 2016;9(1):642.
41. Kidd L, Qurollo B, Lappin M, Richter K, Hart JR, Hill S, et al. Prevalence of vector-borne pathogens in southern California dogs with clinical and laboratory abnormalities consistent with immune-mediated disease. J Vet Intern Med. 2017;31(4):1081-1090.
42. Hackett TB, Jensen WA, Lehman TL, Hohenhaus AE, Crawford PC, Giger U, et al. Prevalence of DNA of Mycoplasma haemofelis, "Candidatus Mycoplasma haemominutum," Anaplasma phagocytophilum, and species of Bartonella, Neorickettsia, and Ehrlichia in cats used as blood donors in the United States. J Am Vet Med Assoc. 2006;229(5):700-705.
43. Díaz-Sánchez AA, Corona-González B, Meli ML, Álvarez DO, Cañizares EV, Rodríguez OF, et al. First molecular evidence of bovine hemoplasma species (Mycoplasma spp .) in water buffalo and dairy cattle herds in Cuba. 2019;1-9.
44. Jaimes-Martínez CA, Quiroz-Castañeda RE, Francisco J, Torre P, Amaro-Estrada I. Detección molecular del hemoplasma Candidatus Mycoplasma haemobos en ganado bovino de México Molecular detection of hemoplasms Candidatus Mycoplasma haemobos in bovine cattle from Mexico. Acta Agrícola y Pecu. 2018;4(3):99-107.
45. McFadden A, Ha HJ, Donald JJ, Bueno IM, van Andel M, Thompson JC, et al. Investigation of bovine haemoplasmas and their association with anaemia in New Zealand cattle. N Z Vet J. 2016;64(1):65-68.
46. Hoelzle K, Winkler M, Kramer MM, Wittenbrink MM, Dieckmann SM, Hoelzle LE. Detection of Candidatus Mycoplasma haemobos in cattle with anaemia. Vet J [Internet]. 2011;187(3):408-410. Available from: http://www.sciencedirect.com/science/article/pii/S1090023310000328
47. Machado CAL, Vidotto O, Conrado FO, Santos NJR, Valente JDM, Barbosa IC, et al. Mycoplasma ovis infection in goat farms from northeastern Brazil. Comp Immunol Microbiol Infect Dis. 2017;55:1-5.
48. Vieira RFC, Molento MB, Santos LC, Moraes W, Cubas ZS, Santos AP. Detection of a novel hemoplasma based on 16S rRNA gene DNA in captive and free-ranging capybaras (Hydrochaeris hydrochaeris). Vet Microbiol. 2009;139. Available from: http://dx.doi.org/10.1016/j.vetmic.2009.06.018
49. Fu Y, Shi T, Xu L, Wei W, Lu F, Zhang X, et al. Identification of a novel Hemoplasma species from pigs in Zhejiang province, China. J Vet Med Sci. 2017; 3;79(5):864-870. Available from: http://

www.ncbi.nlm.nih.gov/pmc/articles/PMC5447974/

50. Aktas M, Ozubek S. A molecular survey of small ruminant hemotropic mycoplasmosis in Turkey, including first laboratory confirmed clinical cases caused by Mycoplasma ovis. Vet Microbiol. 2017;208:217-222.
51. Willi B, Boretti FS, Baumgartner C, Cattori V, Meli ML, Doherr MG. Feline hemoplasmas in Switzerland: identification of a novel species, diagnosis, prevalence, and clinical importance. Schweiz Arch Tierheilkd. 2006;148. Available from: http://dx.doi.org/10.1024/0036-7281.148.3.139
52. Quiroz-Castañeda RE., Aguilar-Díaz H., Preciado De La Torre JF., Salinas-Estrella E. Detección molecular de hemoplasmas que afectan al ganado bovino: Candidatus Mycoplasma haemobos y Mycoplasma wenyonii. Jiutepec, Morelos, México; 2019.

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Authors' contributions: REQC conceived the idea, the intellectual content and coordinated this effort to provide the recent findings of hemoplasmas. REQC and IAE conceptualized the project. HAD performed propidium iodide staining and micrographs of hemoplasmas, REQC, IAE, SRC, HAD wrote the final draft. All authors read and approved the final manuscript.